AMENDMENTS TO THE SPECIFICATION

[0018] Northern analysis of transgenic tobacco plants of the line 35S-DOG. Whole RNA was isolated from tobacco leaves, separated by gel electrophoresis and transferred onto a nylon membrane. Hybridization was performed with a radioactively labeled coding region of the DOG^{R}1 gene. 20 ~~mg~~ µg RNA were applied per lane. Lanes 1-9, independently transformed plants of the line 35S-DOG; lane 10, untransformed control.

[0041] Also, the introduction of point mutations at positions is conceivable in which a change in the amino acid sequence may have an influence on, e.g., the enzyme activity or the regulation of the enzyme. In this way, e.g., mutants with a modified ~~Km~~ K_m value may be produced, or mutants which are no longer subject to the regulatory mechanisms by allosteric regulation or covalent modification usually occurring in cells.

[0082] Plant whole RNA was isolated according to the method by Logemann (1987, Analytical Biochem. 163, 16) and separated on formaldehyde agarose gels (Lehrach (1977) Biochem. 16, 4743). A capillary transfer on nylon membranes (Gene Screen, NEN) was carried out in 20 x SSC (1.5 M NaCl, 150 mM sodium citrate) overnight. After pre-hybridization for two hours in hybridization buffer (500 mM sodium phosphate (pH 7.2), 7% SDS, 1% bovine serum albumin, 200 µg herring sperm DNA, 1 mM EDTA), the hybridization was carried out at 55°C for 16 hours with the radioactively labeled DOG^{R}1 probe. As probe the DOG^{R}1 coding region was isolated from plasmid pGEMT and radioactively labeled in the presence of ~~α-32P-dCTP~~ α-^{32}P-dCTP

using a High Prime Kit (Boehringer). Then, the filters were washed under the following conditions: 20 minutes at 55°C in 6 x SSC, 0.1% SDS and 20 minutes at 55°C in 4 x SSC, 0.1% SDS.

[0085] The DOG^R1 coding region was cloned by Polymerase Chain Reaction (PCR). The matrix material used was genomic yeast DNA which was isolated from Saccharomyces cerevisiae strain S288C according to standard protocol. Amplification was carried out using the specific primers DOG^R1-1 (5'-ATGGATCCCCATGGCAGAATTTTCAGCTGATCTATG-3'; SEQ ID NO:3) and DOG^R1-2 (5' ATGTCGACTACTCAGGCCCTTGTCAAAGGGTTG-3'; SEQ ID NO:4), which were derived from a published sequence (Sanz (1994) Yeast 10, 1195-1202). Primer 1 includes bases 1 to 26 and primer 2 bases 720 to 741 of the coding region of the DOG^R1 gene. For the cloning of the amplified DNA into plant expression vectors the primers additionally carry the following restriction sites: primer 1, BamHI and NcoI; primer 2, SalI. The cloning strategy is depicted in Figure 3. The PCR reaction mixture (50µl) contained chromosomal yeast DNA (1µg), primers 1 and 2 (1µg each), 10 mM Tris-HCl (pH 8.8 at 25°C), 3.5 mM ~~MgCl2~~ $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, 200 µM dNTPs (dATP, dCTP, dGTP, dTTP) and 2 units PrimeZyme DNA Polymerase (Biometra). Before the polymerase was added the mixture was heated to 94°C for 10 minutes. The polymerization steps (60 cycles) were carried out in an automated "Thermocycler" (Perkin Elmer) according to the following program: denaturation at 94°C (1 minute), annealing of the primers at 40°C (1 minute), polymerase reaction at 72°C (1 minute). The fragment obtained was cloned into the vector pGEMT (Promega Corp., 2800 Woods Hollow Road, Madison, WI 53711-5399, USA)

and the plasmid pGEMT- DOG^{R}1 was obtained. The identity of the amplified DNA was verified by sequence analysis.